INVENTORY SUPPLEMENTAL MATERIAL

Figures

Tables

Figure S1

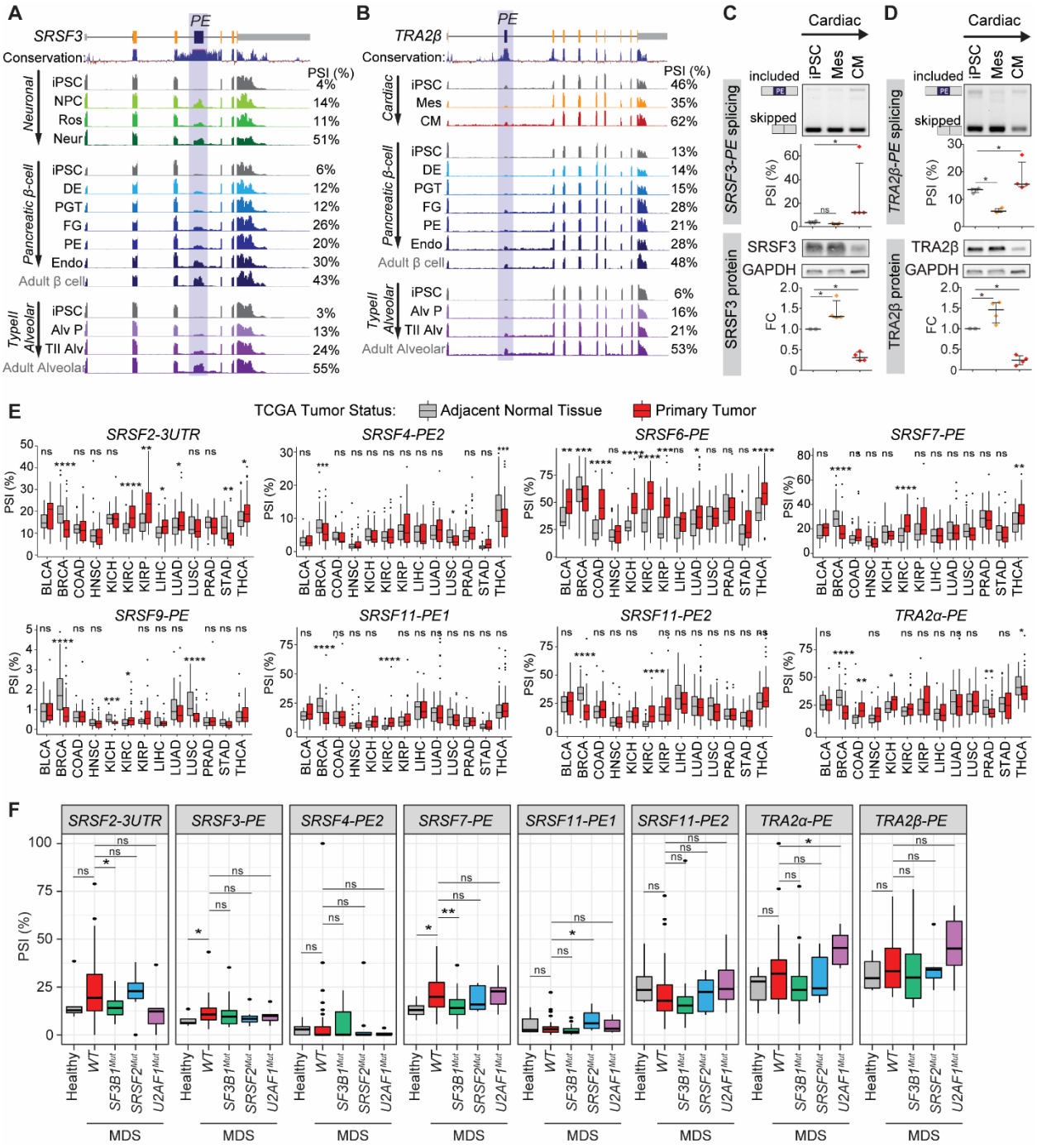

Figure S1. SR protein poison exons are differentially spliced to regulate SR protein expression during iPSC differentiation and in tumors (related to Figure 1).

(A) *SRSF3-PE* inclusion in iPSCs differentiated into neurons, pancreatic β-cells, or Type II alveolar lung epithelial cells visualized using RNA-seq read coverage for the entire gene region. Evolutionary conservation across 100 vertebrates shows the location of ultraconserved regions.

Average exon inclusion (PSI) values from replicates are listed (n≥3 for all timepoints, except n=2 for adult β-cells).

iPSC neuronal (n=18); NPC, neural progenitor cells (n=20); Ros, neural rosette (n=13); Neur, mature neurons (n=4); iPSC pancreatic (n=3); DE, definitive endoderm (n=3); PGT, primitive gut tube (n=3); FG, posterior foregut (n=3); PE, pancreatic endoderm (n=3); Endo, *in vivo* matured endocrine cells (n=4); AdultβC, isolated β-cells from adult patients (n=2); iPSC alveolar (n=3); AlvP, day 15 alveolar epithelial progenitors (n=3); AlvTII, day 35 *in vitro* differentiated type II alveolar epithelial cells(n=3); AdultTII, isolated type II alveolar cells from adult patients (n=3).

(B) Same as in (A) for *TRA2β-PE* inclusion in iPSCs differentiated into cardiomyocytes, pancreatic β-cells, or Type II alveolar lung epithelial cells.

iPSC cardiac (n=4); Mes, Mesoderm (n=4); CM, cardiomyocytes (n=4); iPSC pancreatic (n=3); DE, definitive endoderm (n=3); PGT, primitive gut tube (n=3); FG, posterior foregut (n=3); PE, pancreatic endoderm (n=3); Endo, *in vivo* matured endocrine cells (n=4); AdultβC, isolated β-cells from adult patients (n=2); iPSC alveolar (n=3); AlvP, day 15 alveolar epithelial progenitors (n=3); AlvTII, day 35 *in vitro* differentiated type II alveolar epithelial cells (n=3); AdultTII, isolated type II alveolar cells from adult patients (n=3).

(C,D) *SRSF3-PE* (C) or *TRA2β-PE* (D) inclusion and corresponding protein levels in iPSCs differentiated into mesoderm (Mes) or cardiomyocytes (CM). PE inclusion, quantified as percent spliced in (PSI), is measured by semi-quantitative RT-PCR using primers that amplify both the included and skipped isoforms (n=4, median±interquartile range; Wilcoxon rank test, **P*<0.05). Fold change (FC) protein expression is quantified by western blot using SRSF3 or TRA2β antibodies and GAPDH as a loading control, normalized iPSCs (n=4, median±interquartile range; Wilcoxon rank test, **P*<0.05).

(E) Inclusion of *SR-PEs*, quantified as percent spliced in (PSI), across TCGA tumor types with ≥15 tumor and patient matched adjacent normal tissue samples (n≥30; Wilcoxon rank test, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, ns - not significant). See also Table S1.

BLCA, Bladder urothelial carcinoma (n=39); BRCA, Breast invasive carcinoma (n=213); COAD, Colon adenocarcinoma (n=50); HNSC, Head and neck squamous cell carcinoma (n=84); KICH, Kidney chromophobe (n=45); KIRC, kidney renal clear cell carcinoma (n=132); KIRP, kidney renal papillary cell carcinoma (n=52); LIHC, Liver hepatocellular carcinoma (n=77); LUAD, Lung adenocarcinoma (n=109); LUSC, Lung squamous cell carcinoma (n=96); PRAD, prostate adenocarcinoma (n=104); STAD, Stomach adenocarcinoma (n=50); THCA, Thyroid carcinoma (n=116).

(F) Inclusion of *SR-PEs*, quantified as PSI, in $CD34^{+}$ cells from myelodysplastic syndrome (MDS) patients with SF mutations (*SF3B1*^{Mut} n=28*, SRSF2*^{Mut} n=8*, or U2AF1*^{Mut} n=6), myelodysplastic syndrome patients without SF mutations (*WT*, n=40), or healthy controls (n=8) (Pellagatti et al., 2018) (Wilcoxon rank test, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, ns - not significant). See also Table S1.

Figure S2

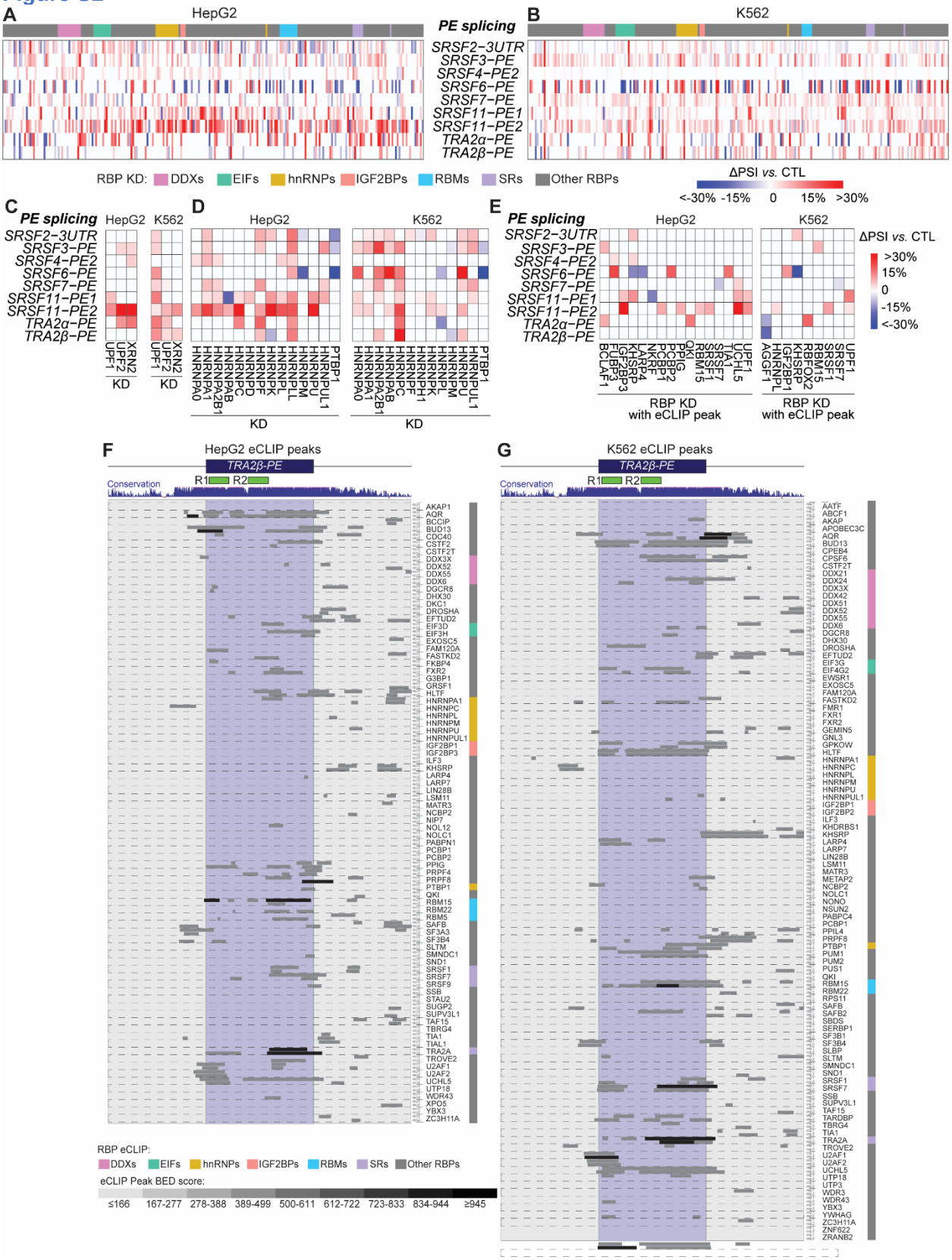
A
HepG2
B
K562
PE splicing
SRSF2−3UTR
SRSF3−PE
SRSF4−PE2
SRSF6−PE
SRSF7−PE
SRSF11−PE1
SRSF11−PE2
TRA2α−PE
TRA2β−PE
RBP KD: DDXs EIFs hnRNPs IGF2BPs RBMs SRs Other RBPs
ΔPSI vs. CTL
<-30% -15% 0 15% >30%
C
PE splicing
HepG2
K562
XRN2
UPF2
UPF1
KD
D
HepG2
K562
PTBP1
HNRNPA0
HNRNPA1
HNRNPA2B1
HNRNPAB
HNRNPC
HNRNPD
HNRNPF
HNRNPK
HNRNPL
HNRNPM
HNRNPU
HNRNPUL1
KD
E
PE splicing
HepG2
K562
ΔPSI vs. CTL
>30%
15%
0
-15%
<-30%
UPF1
UCHL5
TIA1
SRSF7
SRSF1
RBM15
KHSRP
IGF2BP1
HNRNPL
AGGF1
BCLAF1
FUBP3
IGF2BP3
KHSRP
LARP4
NCBP2
PCBP1
PCBP2
PPIG
QKI
RBM15
SRSF7
RBM15
RBM22
RBM5
RBM15
RBM22
RBM5
RBP KD with eCLIP peak
RBP KD with eCLIP peak
F
HepG2 eCLIP peaks
TRA2β-PE
R1
R2
Conservation
RBP eCLIP:
DDXs EIFs hnRNPs IGF2BPs RBMs SRs Other RBPs
eCLIP Peak BED score:
≤166 167-277 278-388 389-499 500-611 612-722 723-833 834-944 ≥945
G
K562 eCLIP peaks
TRA2β-PE
R1
R2
Conservation

Figure S2. Splicing of SR protein poison exons is regulated by multiple RNA binding proteins (related to Figure 2).

(A,B) *SR-PE* inclusion is quantified as Percent spliced-in (PSI) in HepG2 (**A**) or K562 cells (**B**) with SR protein KD (ENCODE). Increased exon inclusion (ΔPSI>0) or skipping (ΔPSI<0) is relative to control (n=2; rMATS, likelihood ratio P>0.05).

(C) *SR-PE* inclusion in HepG2 or K562 cells (ENCODE) with KD of RBPs involved in NMD. Increased exon inclusion (ΔPSI>0) or skipping (ΔPSI<0) is relative to control (n=2; rMATS, likelihood ratio P>0.05).

(D) *SR-PE* inclusion in HepG2 or K562 cells with hnRNP protein KD (ENCODE). Increased exon inclusion (ΔPSI>0) or skipping (ΔPSI<0) is relative to control (n=2; rMATS, likelihood ratio P>0.05).

(E) *SR-PE* inclusion for RBPs that impact splicing in (**A,B**) and bind the SR protein ultraconserved regions as evidenced by the presence of RBP eCLIP peaks in HepG2 or K562 cells (ENCODE). Increased exon inclusion (ΔPSI>0) or skipping (ΔPSI<0) is relative to control (n=2; rMATS, likelihood ratio P>0.05).

(F-G) eCLIP binding peaks from individual RBPs in the *TRA2β-PE* region in HepG2 (**F**) and K562 (**G**) cells (ENCODE). Schematic of *TRA2β-PE* genomic region, the evolutionary conservation across 100 vertebrates, and the locations of region 1 (R1) and region 2 (R2) from Figure 5C are shown. Scores for eCLIP peaks from ENCODE are indicated; peaks with the strongest support are colored in black.

Figure S3

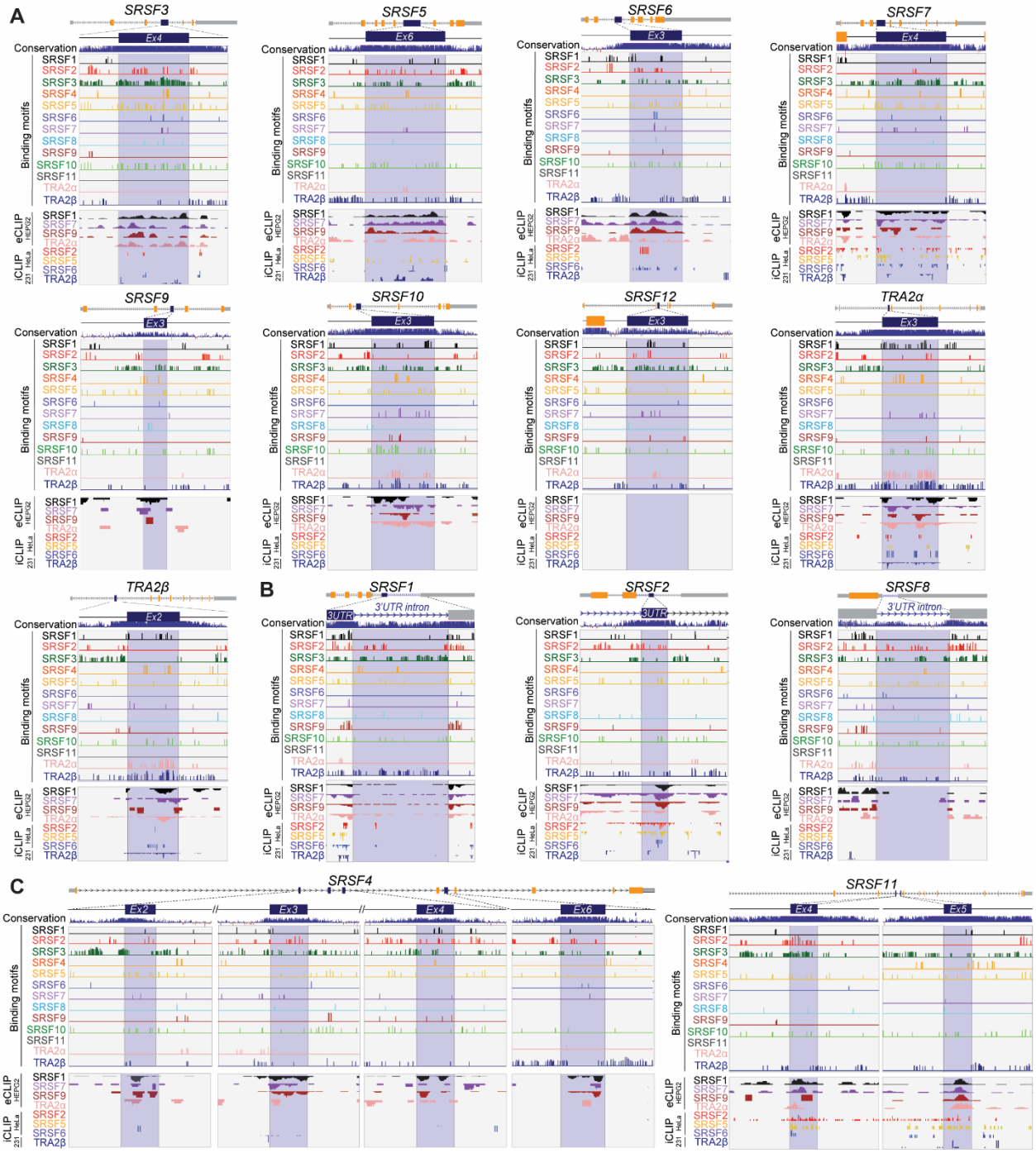

Figure S3. SR protein poison exon sequences contain binding sites for their other protein family members (related to Figure 2).

(A) Predicted SR protein RNA binding motifs and experimental support for binding to *SR-PE* sequences. Schematic of SR protein gene structures 5' to 3' with internal single cassette poison exons (not at scale), along with the evolutionary conservation across 100 vertebrates (top panel). Zoomed in regions shows the location of ultraconserved regions with binding motifs from RBPmap (middle panel) and CLIP-seq read coverage (bottom panel) within the poison exon or poison

sequence (blue). See Table S2 for RBP RNA binding motifs. CLIP-seq data from HepG2 and K562 cells (ENCODE datasets), as well as HeLa and MDA-MB231 (Best et al., 2014; Krchnakova et al., 2019; Van Nostrand et al., 2016).

(B,C) Same as in (**A**) for SR proteins with 3'UTR poison sequences (B) or with multiple PEs (C).

Figure S4

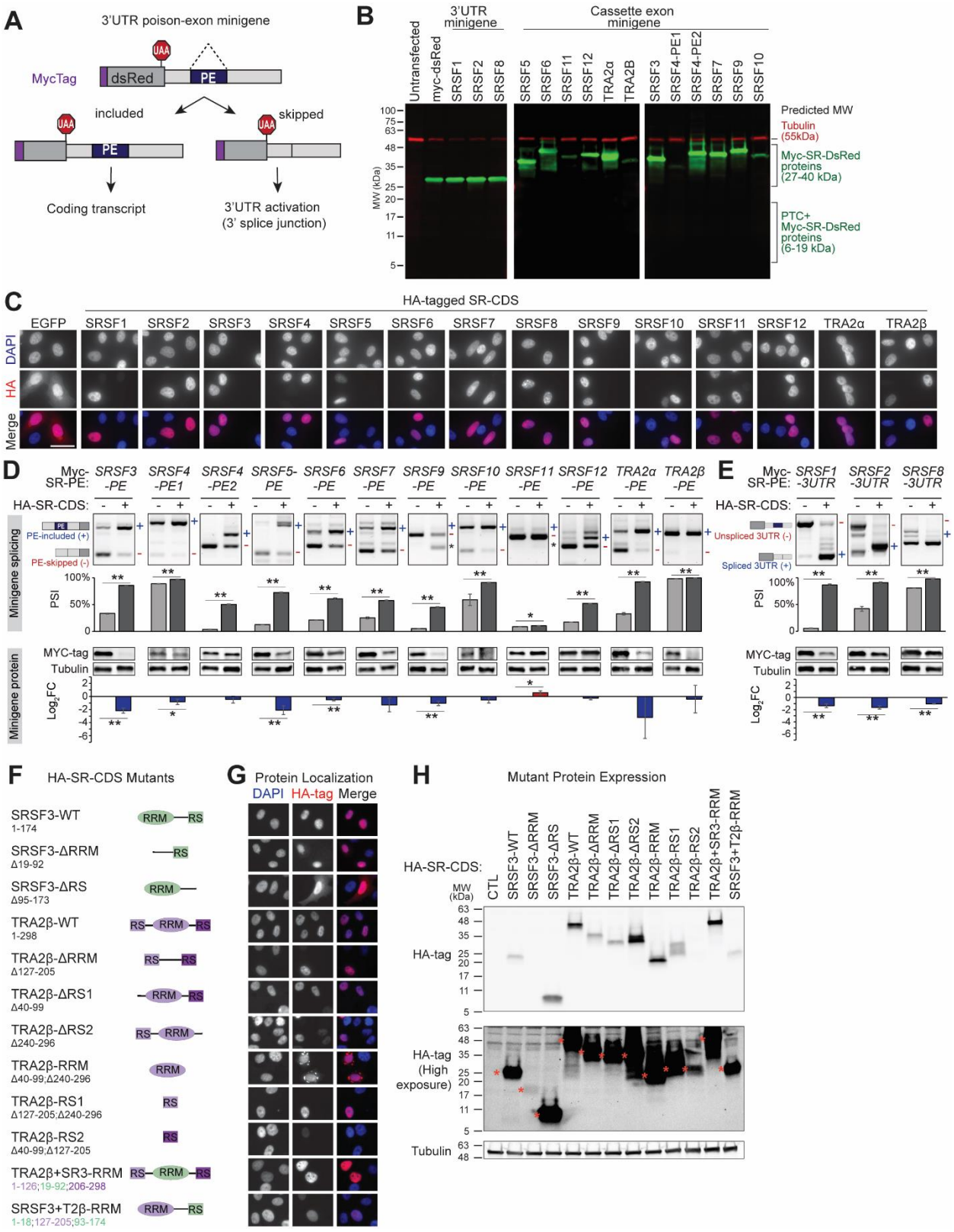
A
3'UTR poison-exon minigene
MycTag
dsRed
PE
UAA
included
skipped
Coding transcript
3'UTR activation
(3' splice junction)
B
3'UTR minigene
Cassette exon minigene
Untransfected
myc-dsRed
SRSF1
SRSF2
SRSF8
SRSF5
SRSF6
SRSF11
SRSF12
TRA2α
TRA2B
SRSF3
SRSF4-PE1
SRSF4-PE2
SRSF7
SRSF9
SRSF10
MW (kDa)
Predicted MW
Tubulin (55kDa)
Myc-SR-DsRed proteins (27-40 kDa)
PTC+ Myc-SR-DsRed proteins (6-19 kDa)
C
HA-tagged SR-CDS
EGFP
SRSF1
SRSF2
SRSF3
SRSF4
SRSF5
SRSF6
SRSF7
SRSF8
SRSF9
SRSF10
SRSF11
SRSF12
TRA2α
TRA2β
DAPI
HA
Merge
D
Myc-SR-PE:
SRSF3-PE
SRSF4-PE1
SRSF4-PE2
SRSF5-PE
SRSF6-PE
SRSF7-PE
SRSF9-PE
SRSF10-PE
SRSF11-PE
SRSF12-PE
TRA2α-PE
TRA2β-PE
HA-SR-CDS:
Minigene splicing
PE-included (+)
PE-skipped (-)
PSI
Minigene protein
MYC-tag
Tubulin
Log2FC
E
Myc-SR-PE:
SRSF1-3UTR
SRSF2-3UTR
SRSF8-3UTR
HA-SR-CDS:
Unspliced 3UTR (-)
Spliced 3UTR (+)
PSI
MYC-tag
Tubulin
Log2FC
F
HA-SR-CDS Mutants
SRSF3-WT
1-174
SRSF3-ΔRRM
Δ19-92
SRSF3-ΔRS
Δ95-173
TRA2β-WT
1-298
TRA2β-ΔRRM
Δ127-205
TRA2β-ΔRS1
Δ40-99
TRA2β-ΔRS2
Δ240-296
TRA2β-RRM
Δ40-99;Δ240-296
TRA2β-RS1
Δ127-205;Δ240-296
TRA2β-RS2
Δ40-99;Δ127-205
TRA2β+SR3-RRM
1-126;19-92;206-298
SRSF3+T2β-RRM
1-18;127-205;93-174
RRM
RS
G
Protein Localization
DAPI HA-tag Merge
H
Mutant Protein Expression
HA-SR-CDS:
CTL
SRSF3-WT
SRSF3-ΔRRM
SRSF3-ΔRS
TRA2β-WT
TRA2β-ΔRRM
TRA2β-ΔRS1
TRA2β-ΔRS2
TRA2β-RRM
TRA2β-RS1
TRA2β-RS2
TRA2β+SR3-RRM
SRSF3+T2β-RRM
MW (kDa)
HA-tag
HA-tag (High exposure)
Tubulin

Figure S4. SR proteins use poison exons to auto- and cross-regulate their protein expression (related to Figures 2 and 4).

(A) Schematic of the splicing reporter minigene for 3'UTR poison sequences and resulting processed isoforms. Sequences encoding the Myc-epitope tag, dsRed protein, and stop codon locations are indicated. For *SRSF1* and *SRSF8,* removal of 3'UTR introns results in formation of a splice junction after the canonical stop codon. For *SRSF2,* the AS-NMD transcript requires removal of 3'UTR introns as well as inclusion of a 3'UTR exon.

(B) Representative western blot analysis of Myc-tagged proteins expressed from *SR-PE* minigenes in HEK293 cells using a minigene specific Myc-tag antibody (green) and tubulin (red) as a loading control. Minigenes are grouped by 3'UTR or cassette exons presence, as well as based on protein expression for cassette exon minigenes. SR protein minigenes produce one discrete band on western blot with no detectable truncated products from potential translation of premature termination codon (PTC+)-containing transcripts. All three SR protein 3'UTR minigenes produce a 27kDa Myc-tagged Ds-Red-containing protein, whereas cassette exon minigenes produce Myc-tagged Ds-Red-containing proteins ranging in predicted molecular weight (MW) between 27-45kDa.

(C) Representative immunofluorescence images of HeLa cells transfected with HA-CDS-SR expression plasmids, stained with mouse anti-HA antibody and Alexa568 secondary antibody. Nuclei are counterstained with DAPI (scale, 30µm).

(D) PE splicing and protein expression from HEK293 cells co-transfected with cassette PE minigenes along with HA-CDS-SR plasmid for the corresponding SR protein or empty vector control. Exon inclusion is quantified using RT-PCR with minigene specific primers that amplifies both exon skipping and included isoforms. Increased exon inclusion (ΔPSI>0) or skipping (ΔPSI<0) is relative to empty vector control (n=3, mean±sd; t-test, **P*<0.05, ***P*<0.01). Log_2 Fold change (Log_2FC) protein expression is quantified by western blot using a minigene specific Myc-tag antibody and tubulin as a loading control, normalized to empty vector control (n=3, mean±sd; t-test, **P*<0.05, ***P*<0.01).

Novel examples of validated autoregulation include: SRSF4 which contains two sets of PEs, either exons 2, 3 and 4 which are included together (*SRSF4-PE1)* or exon 6 alone (*SRSF4-PE2)*. Previous studies have shown that exogenous SRSF4 expression can decrease the endogenous protein expression and that SRSF4 directly binds to its PEs through CLIP-seq, but have not shown this interaction to be directly attributed to splicing of its PE (Anko et al., 2010; Anko et al., 2012).

Additionally, we identify autoregulation of PE inclusion in *SRSF6*, *SRSF10*, *SRSF12*, and *TRA2α*, and of 3'UTR poison sequences in *SRSF8*. Notably, the *SRSF9* and *SRSF11* minigenes undergo alternative splicing to create transcripts that contain a frameshift due to skipping of both the PE and adjacent downstream exon (denoted with an asterisk *). This creates a frameshift and premature termination codon (PTC) in the *SRSF9-PE* minigene but not in the *SRSF11-PE*. However, both of these splicing events lead to frameshift-induced PTCs in the endogenous gene.

The *SRSF12-PE* minigene contains five total exons to recapitulate the minor and major spliceosome sites across these exons in the endogenous context. Splicing of *SRSF12-PE* leads to 3 distinct isoforms detected by PCR: i) the top band contains the PE and next downstream exon that utilizes an alternative 3'SS corresponding to the major spliceosome; ii) the middle band contains the PE and skips the next downstream exon; iii) the bottom band skips the PE and contains all of the coding sequence exons. The top and middle bands are included for quantification of *PE* PSI.

(E) Same as in (**D**) for SR protein 3'UTR minigenes. AS-NMD events in the UTR of SRSF1, SRSF2, and SRSF8 are more complex than those of cassette PEs. For SRSF1, alternative splicing of the 3'UTR involves multiple intron removals that flank ultraconserved regions and

multiple splicing events can be induced by SRSF1 overexpression and stabilized by NMD inhibition (Lareau et al., 2007; Sun et al., 2010). We confirmed our SRSF1-3UTR minigene recapitulates its reported autoregulatory splicing through sanger sequencing, however the minigene uses a 5' splice site (5'SS) located 14bp downstream of the 3'UTR 5'SS sequence and 4bp into the dsRed sequence, and which is predicted to have similar strength as the endogenous 5'SS. The SRSF2-3UTR minigene autoregulation utilized inclusion of an exonic sequence within the 3'UTR as described for the endogenous gene (Lareau et al., 2007; Sureau et al., 2001).

(F) Domain structure of wild-type and mutant SRSF3 and TRA2β proteins. Included or deleted (Δ) amino acids positions are indicated. RRM: RNA-recognition motif; RS: arginine-serine rich domain.

(G) Representative immunofluorescence images of HeLa cells transfected with 500ng of HA-CDS-SR expression plasmids from (A), stained with mouse anti-HA antibody and Alexa568 secondary antibody. Nuclei are counterstained with DAPI (scale, 30um).

(H) Expression of HA-CDS-SR expression plasmids from (A) in HeLa cells detected by western blotting with anti-HA antibody and tubulin as a loading control. Bands corresponding to the expected protein molecular weight (MW) are marked with orange asterisk (*).

Figure S5

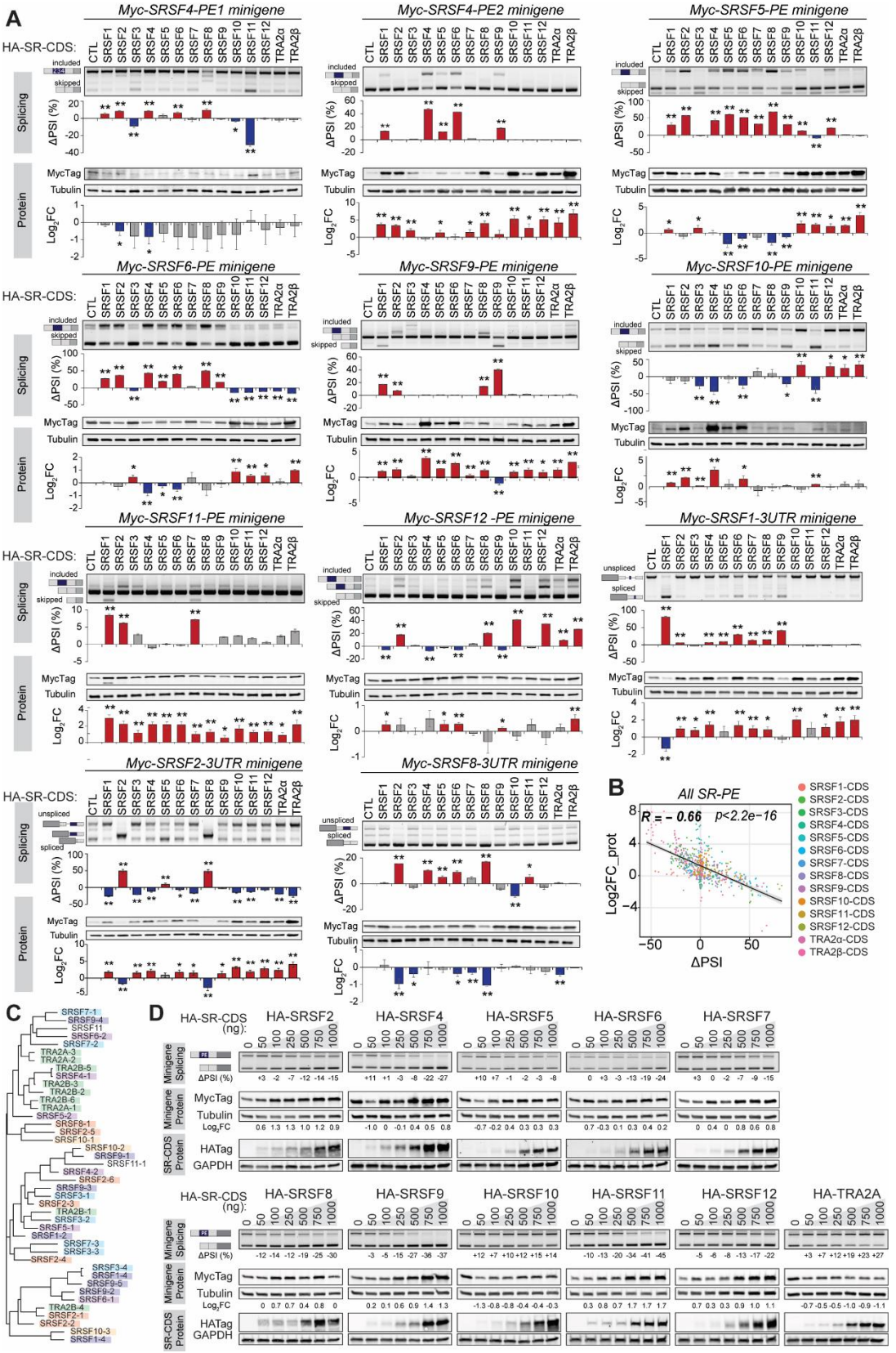
A
Myc-SRSF4-PE1 minigene
Myc-SRSF4-PE2 minigene
Myc-SRSF5-PE minigene
Myc-SRSF6-PE minigene
Myc-SRSF9-PE minigene
Myc-SRSF10-PE minigene
Myc-SRSF11-PE minigene
Myc-SRSF12 -PE minigene
Myc-SRSF1-3UTR minigene
Myc-SRSF2-3UTR minigene
Myc-SRSF8-3UTR minigene
HA-SR-CDS:
CTL SRSF1 SRSF2 SRSF3 SRSF4 SRSF5 SRSF6 SRSF7 SRSF8 SRSF9 SRSF10 SRSF11 SRSF12 TRA2α TRA2β
Splicing
Protein
included
skipped
unspliced
spliced
ΔPSI (%)
MycTag
Tubulin
Log2FC
B
All SR-PE
R = - 0.66 p<2.2e-16
Log2FC_prot
ΔPSI
SRSF1-CDS
SRSF2-CDS
SRSF3-CDS
SRSF4-CDS
SRSF5-CDS
SRSF6-CDS
SRSF7-CDS
SRSF8-CDS
SRSF9-CDS
SRSF10-CDS
SRSF11-CDS
SRSF12-CDS
TRA2α-CDS
TRA2β-CDS
C
D
HA-SR-CDS (ng):
HA-SRSF2
HA-SRSF4
HA-SRSF5
HA-SRSF6
HA-SRSF7
HA-SRSF8
HA-SRSF9
HA-SRSF10
HA-SRSF11
HA-SRSF12
HA-TRA2A
Minigene Splicing
Minigene Protein
SR-CDS Protein
ΔPSI (%)
MycTag
Tubulin
Log2FC
HATag
GAPDH

Figure S5. SR proteins cross-regulate their expression in a dose-dependent manner through alternative splicing of poison exons (related to Figure 2-3).

(A) PE splicing and protein expression is measured by semi-quantitative RT-PCR using minigene specific primers that amplify both the included and skipped isoforms. Increased exon inclusion (ΔPSI>0) or skipping (ΔPSI<0) is relative to empty vector control (n=3, mean±sd; t-test, **P*<0.05, ***P*<0.01). Log_2 Fold change (Log_2FC) protein expression is quantified by western blot using a minigene specific Myc-tag antibody and tubulin as a loading control, normalized to empty vector control (n=3, mean±sd; t-test, **P*<0.05, ***P*<0.01).

(**B**) Pearson correlation between SR protein minigene splicing (ΔPSI) and SR protein expression (Log_2FC) aggregated for all SR-PEs in cells transfected with indicated SR-CDS, as quantified in Figures 2C-F and S5A. *P*-values are indicated. See also Table S3.

(**C**) Clustering of SR protein motifs based on sequence similarity. Related SR protein family members are similarly colored.

(**D**) PE splicing and protein expression from the *TRA2β-PE* minigene in HeLa cells cotransfected with and increasing concentration of indicated HA-SR-CDS plasmids (supplemented up to 1000ng of DNA with control empty vector). *TRA2β-PE* inclusion is measured by semi-quantitative RT-PCR using minigene specific primers that amplify both the included and skipped isoforms. Increased exon inclusion (ΔPSI>0) or skipping (ΔPSI<0) is relative to empty vector control. Log_2 Fold change (Log_2FC) protein expression is quantified by western blotting using a minigene specific Myc-tag antibody and tubulin as a loading control, normalized to empty vector control. HA-SR-CDS protein expression is detected using HA-tag antibody and GAPDH as a loading control. See also heatmap representation of PE splicing and protein levels in Figure 3B.

Figure S6

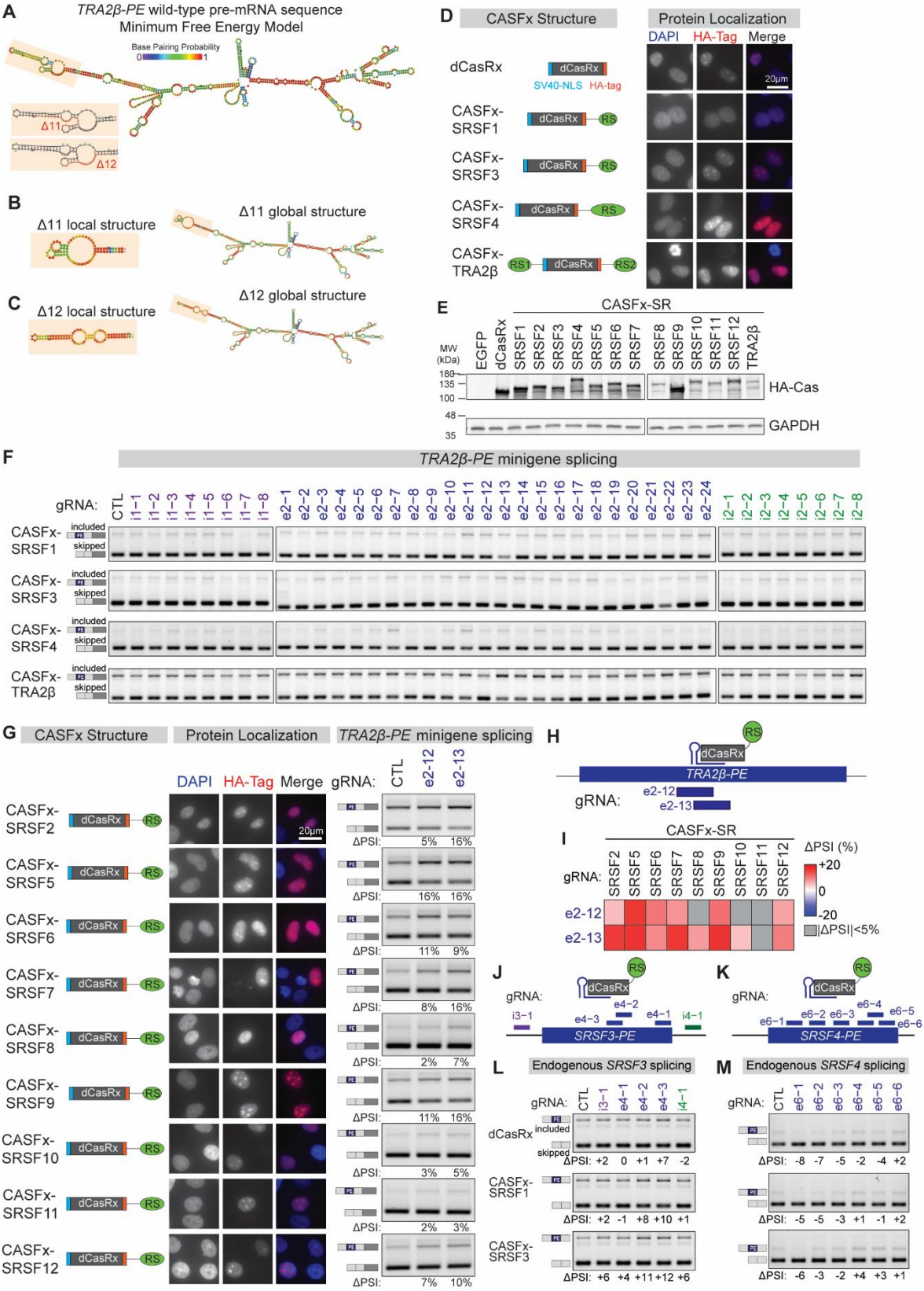
A
TRA2β-PE wild-type pre-mRNA sequence
Minimum Free Energy Model
Base Pairing Probability
Δ11
Δ12
B
Δ11 local structure
Δ11 global structure
C
Δ12 local structure
Δ12 global structure
D
CASFx Structure
Protein Localization
DAPI
HA-Tag
Merge
dCasRx
SV40-NLS
HA-tag
CASFx-SRSF1
CASFx-SRSF3
CASFx-SRSF4
CASFx-TRA2β
RS
RS1
RS2
20μm
E
CASFx-SR
MW (kDa)
EGFP
dCasRx
SRSF1
SRSF2
SRSF3
SRSF4
SRSF5
SRSF6
SRSF7
SRSF8
SRSF9
SRSF10
SRSF11
SRSF12
TRA2β
180
135
100
48
35
HA-Cas
GAPDH
F
TRA2β-PE minigene splicing
gRNA:
CTL
CASFx-SRSF1
CASFx-SRSF3
CASFx-SRSF4
CASFx-TRA2β
included
skipped
G
CASFx Structure
Protein Localization
TRA2β-PE minigene splicing
CASFx-SRSF2
CASFx-SRSF5
CASFx-SRSF6
CASFx-SRSF7
CASFx-SRSF8
CASFx-SRSF9
CASFx-SRSF10
CASFx-SRSF11
CASFx-SRSF12
ΔPSI:
H
TRA2β-PE
I
CASFx-SR
ΔPSI (%)
+20
0
-20
|ΔPSI|<5%
J
SRSF3-PE
K
SRSF4-PE
L
Endogenous SRSF3 splicing
M
Endogenous SRSF4 splicing

Figure S6. Defining the splicing regulatory elements and splicing regulators of *TRA2β-PE* inclusion (related to Figures 5, 6).

(A) Predicted minimum free energy structure of wild-type *TRA2β-PE* and 250bp of flanking intron sequences using RNA fold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) (Lorenz et al., 2011). The *TRA2β-PE* Δ11 and Δ12 region is highlighted.

(B-C) Predicted minimum free energy structure of *TRA2β-PE* and 250bp of flanking intron for the *TRA2β-PE* Δ11 (**B**) and Δ12 (**C**) deletion mutants.

(D) Structure and localization of HA-tagged CASFx-SR proteins. Diagrams indicating CASFx-SR protein structures consisting of N-terminal dCasRx and C-terminal RS domain for each SR protein (SRSF1-12), or N-terminal RS1 domain followed by dCasRx and C-terminal RS2 domain for TRA2β. Representative immunofluorescence images of HeLa cells transfected with 500ng of CASFx-SR proteins plasmids, stained with mouse anti-HA antibody and Alexa-568 secondary antibody. Nuclei are counterstained with DAPI (scale, 20μm).

(E) Protein expression HA-tagged CASFx-SR proteins transfected in HEK293T cells is assessed at 48h post transfection by western blotting with an HA-tag antibody and tubulin as a loading control.

(F) *TRA2β-PE* inclusion is assessed using RT-PCR with minigene specific primers and normalized as ΔPSI to non-targeting control gRNA (CTL) as summarized in the heatmap Figure 6C.

(G) Structure and localization of additional CASFx-SR proteins as in (**D**) *TRA2β-PE* splicing assessed by cotransfecting HEK293T cells with the *TRA2β-PE* minigene, CASFx-SR protein plasmid, and indicated gRNA plasmid. *TRA2β-PE* inclusion is measured by semi-quantitative RT-PCR using minigene specific primers that amplify both the included and skipped isoforms, and normalized to non-targeting control gRNA (CTL). Binding positions of the 22nt gRNAs targeting the *TRA2β* poison exon (e2-12 and e2-13) are indicated (E).

(H) Target sites for 22nt gRNAs in dCasRx and CASFx-SR proteins in *SRSF3-PE* and flanking introns.

(I) Heatmap representation of *TRA2β-PE* splicing from (**D**) as ΔPSI for each targeting gRNA *vs.* non-targeting control gRNA (CTL).

(J,L) PE inclusion in the endogenous *SRSF3* transcript is measured in HEK293T cells transfected with indicated dCasRx or CASFx-SR plasmids and gRNAs targeting *SRSF3-PE* or surrounding introns. *SRSF3-PE* inclusion assessed using RT-PCR with gene specific primers and normalized as ΔPSI to non-targeting control gRNA (CTL).

(K,M) Same as in (**J,L**) with the endogenous *SRSF4* transcript.

Figure S7

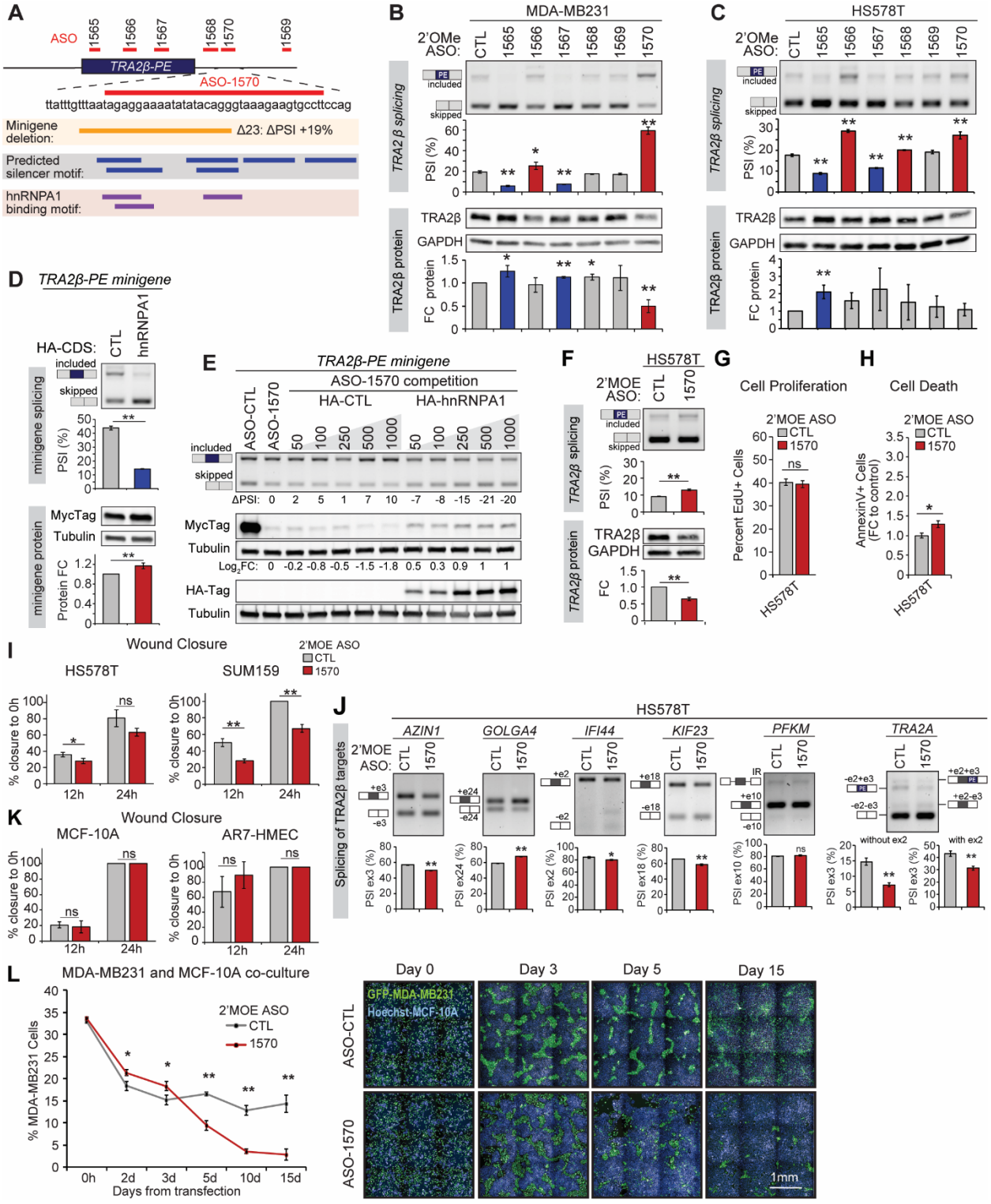
A
ASO
1565
1566
1567
1568
1570
1569
TRA2β-PE
ASO-1570
ttatttgtttaatagaggaaaatatatacagggtaaagaagtgccttccag
Minigene deletion:
Δ23: ΔPSI +19%
Predicted silencer motif:
hnRNPA1 binding motif:
B
MDA-MB231
2'OMe ASO:
CTL
TRA2β splicing
included
skipped
PSI (%)
TRA2β protein
TRA2β
GAPDH
FC protein
C
HS578T
D
TRA2β-PE minigene
HA-CDS:
hnRNPA1
minigene splicing
minigene protein
MycTag
Tubulin
Protein FC
E
TRA2β-PE minigene
ASO-1570 competition
ASO-CTL
ASO-1570
HA-CTL
HA-hnRNPA1
ΔPSI:
Log2FC:
HA-Tag
F
HS578T
2'MOE ASO:
G
Cell Proliferation
2'MOE ASO
Percent EdU+ Cells
ns
H
Cell Death
AnnexinV+ Cells (FC to control)
I
Wound Closure
HS578T
SUM159
% closure to 0h
12h
24h
J
HS578T
AZIN1
GOLGA4
IFI44
KIF23
PFKM
TRA2A
Splicing of TRA2β targets
without ex2
with ex2
K
Wound Closure
MCF-10A
AR7-HMEC
L
MDA-MB231 and MCF-10A co-culture
% MDA-MB231 Cells
Days from transfection
Day 0
Day 3
Day 5
Day 15
ASO-CTL
ASO-1570
GFP-MDA-MB231
Hoechst-MCF-10A
1mm

Figure S7. *TRA2β*-targeting ASOs impact *TRA2β-PE* splicing as well as TRA2β protein expression and activity (related to Figure 7).

(A) Binding locations of ASOs targeting *TRA2β-PE* and the downstream intron. The bottom panel shows the binding sequence of ASO-1570 along with the location of Δ23 deletion from Figure 5, as well as splicing silencer and HNRNPA1 binding motifs predicted by Human Splicing Finder (Desmet et al., 2009).

(B-C) *TRA2β-PE* splicing in MDA-MB231 (**B**) and HS578T (**C**) cells transfected with 50nM of *TRA2β*-targeting or non-targeting control (CTL) 2'-O-methyl-phosphorothioated (2'OMe) ASOs. *TRA2β-PE* inclusion and TRA2β protein expression are measured with RT-PCR and western blot, respectively, 48h after transfection (n=3, mean±SD; t-test, **P*<0.05, ***P*<0.01).

(D) HeLa cells cotransfected with the *TRA2β-PE* minigene and either a control plasmid (CTL) or one encoding an HA-tagged hnRNPA1-CDS. Minigene splicing and protein production assessed by RT-PCR and western blotting 48h after transfection (n=3, mean±SD; t-test, **P*<0.05, ***P*<0.01).

(E) HeLa cells cotransfected with the *TRA2β-PE* minigene along with *TRA2β*-targeting (1570) or control (CTL) ASO alone, or with increasing amounts of HA-hnRNPA1-CDS or control plasmids. Minigene splicing and protein production assessed by RT-PCR and western blotting 48h after transfection, compared to ASO-1570 alone.

(F) *TRA2β-PE* splicing and TRA2β protein expression in HS578T cells transfected with 50nM of ASO-1570 (n=3, mean±SD; t-test, **P*<0.05, ***P*<0.01).

(G) Cell proliferation in HS578T breast cancer cells transfected with 50nM of 2'MOE ASO-1570 *vs.* -CTL is quantified by 5-Ethynyl-2´-deoxyuridine (EdU) incorporation 48h after transfection. Representative stains for Alexa 647 EdU and Hoechst in MDA-MB231 cells are shown in the left panel. The percent of EdU+ cells to total Hoechst+ cells is shown (n=3, mean±SD; t-test, **P*<0.05, ***P*<0.01).

(H) Cell death in HS578T breast cancer cells transfected with 50nM of 2'MOE ASO-1570 *vs.* -CTL is quantified by AnnexinV staining 48h after transfection. Representative stains for Alexa 647 conjugated AnnexinV and Hoechst in MDA-MB231 and SUM159 cells are shown in the left panel. The percent of AnnexinV+ cells to total Hoechst+ cells is normalized to ASO-CTL (n=3, mean±SD; t-test, **P*<0.05, ***P*<0.01).

(I) Wound closure assays in HS578T and SUM159 cells transfected with 50nM of 2'MOE ASO-1570 *vs.* -CTL. Percent wound closure is relative to 0h (n=3, mean±SD; t-test,**P*<0.05, ***P*<0.01).

(J) Splicing of known TRA2β target transcripts is measured in HS578T cells transfected with 50nM of 2'-O-methoxyethyl-phosphorothioated (2'MOE) ASO-1570 *vs.* -CTL, 48h after ASO transfection. Exon inclusion is measured by RT-PCR (n=3, mean±SD; t-test **P*<0.05, ***P*<0.01).

(K) Wound closure assays in MCF-10A and AR7-HMEC non-transformed mammary epithelial cells transfected with 50nM of 2'MOE ASO-1570 *vs.* -CTL. Percent wound closure is relative to 0h (n=3,mean±SD; t-test,**P*<0.05, ***P*<0.01).

(L) GFP-tagged MDA-MB231 co-cultured with MCF-10A cells are transfected with 50nM of 2'MOE ASO-1570 *vs.* -CTL. Cell number is quantified at various timepoints by live cell imaging with GFP and Hoechst stain. The proportion of MDA-MB231 cells is calculated as number of GFP+ cells divided by total number of Hoechst+ nuclei (n=3, mean±SD; t-test, **P*<0.05, ***P*<0.01).